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**Hepatocellular carcinoma: Therapeutic advances in signaling, epigenetic and immune targets**

Neureiter D *et al*. HCC in research and clinical practice

Daniel Neureiter, Sebastian Stintzing, Tobias Kiesslich, Matthias Ocker

**Daniel Neureiter,** Institute of Pathology, Cancer Cluster Salzburg, Paracelsus Medical University/Salzburger Landeskliniken (SALK), Salzburg 5020, Austria

**Sebastian Stintzing,** Medical Department, Division of Oncology and Hematology, Campus Charité Mitte, Charité University Medicine Berlin, Berlin 10117, Germany

**Tobias Kiesslich,** Department of Internal Medicine I, Paracelsus Medical University/Salzburger Landeskliniken (SALK) and Institute of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg 5020, Austria

**Matthias Ocker,** Translational Medicine Oncology, Bayer AG, Berlin 13353, Germany

**Matthias Ocker,** Charité University Medicine Berlin, Berlin 10117, Germany

**ORCID number:** Daniel Neureiter (0000-0001-9155-5762); Sebastian Stintzing (0000-0002-3297-5801); Tobias Kiesslich (0000-0001-5403-9478); Matthias Ocker (0000-0001-8263-6288).

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**Corresponding author:** **Matthias Ocker, MD, Professor,** Translational Medicine Oncology, Bayer AG, Muellerstrasse 178, Berlin 13353, Germany. matthias.ocker@bayer.com

**Telephone:** +49-30-468194799

**Fax:** +49-30-468994799

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**Abstract**

Hepatocellular carcinoma (HCC) remains a global medical burden with rising incidence due to chronic viral hepatitis and non-alcoholic fatty liver diseases. Treatment of advanced disease stages is still unsatisfying. Besides first and second generation tyrosine kinase inhibitors, immune checkpoint inhibitors have become central for the treatment of HCC. New modalities like epigenetic therapy using histone deacetylase inhibitors (HDACi) and cell therapy approaches with chimeric antigen receptor T cells (CAR-T cells) are currently under investigation in clinical trials. Development of such novel drugs is closely linked to the availability and improvement of novel preclinical and animal models and the identification of predictive biomarkers. The current status of treatment options for advanced HCC, emerging novel therapeutic approaches and different preclinical models for HCC drug discovery and development are reviewed here.

**Key words:** Liver cancer; Immunotherapy; Checkpoint inhibitors; Targeted therapy; Mouse model; Biomarker; Next-generation sequencing; Non-alcoholic steatohepatitis; Fibrosis; Clinical trial

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**Core tip:** Treatment of advanced hepatocellular carcinoma still represents an unmet medical need. Novel therapeutic options comprise new tyrosine kinase inhibitors, epigenetic modifiers and increasingly also cell therapy and immune checkpoint inhibitors and combinations of those modalities. Development of better drugs is closely linked to improved preclinical and animal models and has to be accompanied by the implementation of predictive biomarkers, which is still lacking for hepatocellular carcinoma. The current status of these aspects is reviewed in this manuscript.

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**INTRODUCTION**

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver, accounting for approximately 85% of all cases. It is considered to be the 6th most commonly diagnosed cancer and the 4th most common cause of cancer related death worldwide, with 2 to 3 times higher rates for men[1]. Major risk factors are chronic viral hepatitis [hepatitis B virus (HBV) and hepatitis C virus (HCV)], aflatoxin exposure, alcohol intake, and the globally increasing high rates of obesity and type 2 diabetes[2]. The 5-year survival rate of advanced HCC remains devastating at 1% and is the poorest of all solid cancers[3]. Treatment of advanced stages of HCC is often limited by the underlying liver disease which is commonly accompanied by cirrhosis and end-stage liver disease with significantly impaired liver function. Due to those different etiologies and pathogenetic mechanisms, the identification of common oncogenic drivers is challenging although heterogeneous sets of mutations could be detected in HCC (Table 1), of which especially telomerase, p53, β-catenin and others were linked to distinct and prognostic HCC subtypes (Table 2)[4,5].

Curative therapy is currently only possible in early stages by complete surgical resection or orthotopic liver transplantation, the latter being limited by availability of donor organs. Locoregional therapies [*e.g.*, transarterial chemoembolization (TACE), different ablation strategies, selective internal radiotherapy (SIRT)] are available for intermediate stages or HCCs not amenable to surgical therapy and can be applied repeatedly also for downstaging in preparation of transplantation or in an adjuvant setting prior to surgery[6,7]. In addition, external beam radiation (EBRT) is a valuable adjuvant therapy option for small HCCs, in combination with surgery or other locoregional therapies or as a bridging option to orthotopic liver transplantation. It can also help to reduce pain in extrahepatic metastases and prolong survival after surgical resection[8]. For further details on locoregional and non-systemic treatment options, we refer the reader to a recent meta-analysis on the management of HCC[9].

Since the introduction of the multi-kinase inhibitor sorafenib about a decade ago, only little progress has been made in treatment of advanced HCC. In this article, we will review the current status of novel drugs for the treatment of advanced HCC including the emerging immune checkpoint inhibitor therapies. We will also highlight recent trends in identifying predictive biomarkers and establishing animal models that closely resemble the complex and diverse human pathophysiology of HCC.

**Conventional chemotherapy and molecular targeted therapies**

Prior to the approval of sorafenib, no standard chemotherapy regimen had been established for treatment of advanced HCC. Randomized trials and meta-analyses showed a poor response rate of different agents like 5-fluorouracil (5-FU), cisplatin, doxorubicin or hormonal therapy (*e.g.*, tamoxifen or somatostatin-analogues). The high intrinsic resistance of HCC is considered due to the high expression of efflux pumps (linked to the physiologic metabolic capacity of the liver parenchyma), altered blood flow and fibrosis as well as high expression and mutations in drug resistance genes like p53. Most tested drugs showed only modest activity with minimal improvement in overall survival but with significant toxicities in combination[6,10].

The small-molecule multi-kinase inhibitor sorafenib was the first drug to show an overall survival benefit in first-line therapy of advanced HCC (10.7 mo *vs* 7.9 mo; 6.5 mo *vs* 4.2 mo in Asian patients) in randomized controlled trials[11,12]. Since then, only lenvatinib was able to achieve increased overall survival (in a phase 2 study) and was proven to be non-inferior to sorafenib in a recent phase 3 study, reaching a median survival time of 13.6 mo compared to 12.3 mo for sorafenib[13,14].

Similarly, results for new drugs in a second-line setting after failure of sorafenib were mostly disappointing. Regorafenib, a derivative of sorafenib, and the novel multi-kinase inhibitor cabozantinib achieved a significant increase in overall survival in placebo-controlled trials. Regorafenib increased overall survival to 10.6 mo *vs* 7.8 mo[15], while cabozantinib achieved overall survival of 10.2 mo *vs* 8.0 mo with toxicity similar to regorafenib[16]. Lately the vascular endothelial growth factor receptor 2 (VEGFR-2) antibody ramucirumab proved efficacy in sorafenib pretreated patients with an alpha-fetoprotein level of ≥ 400 mg/mL[17]. The randomized phase-III placebo controlled trial REACH-2 showed median overall survival times of 8.5 mo for ramucirumab treated patients *vs* 7.3 mo for the placebo arm [hazard ratio (HR) = 0.7; *P* < 0.0001]. This is the first study to show a significant survival benefit for a biomarker (alpha-fetoprotein) selected subgroup of HCC.

Several other targets for inhibition of receptor tyrosine kinase function in HCC were investigated. Hepatocyte growth factor (HGF) and its receptor c-Met are commonly overexpressed in HCC and have been linked to poor prognosis and resistance to *e.g.*, sorafenib treatment[18-20]. c-Met is targeted by several multi-kinase inhibitors like gefitinib or cobazitinib and more recently also selective inhibitors like capmatinib or tepotinib entered clinical trials but results for studies in HCC are still pending[21-23]. Other less selective compounds with c-Met inhibition properties like crizotinib, brivanib or foretinib did not lead to significant prolongation of overall survival (OS) in phase III studies or were not investigated in HCC patients yet[21,22,24,25].

All of these compounds are recommended for patients with preserved liver function, *i.e.*, Child-Pugh score 5, 6 and 7[26]. Treatment options for patients with more advanced liver impairment or cirrhosis are still lacking and represent an urgent medical need. Esp. the use of sorafenib in patients with portal vein tumor thrombosis (PVTT) remains controversial[27]. In a study with 30 patients with advanced HCC and PVTT treated with sorafenib monotherapy, a disease control rate of 33.3% was achieved, including thrombus revascularization in a small number of patients. Yet, OS and progression-free survival (PFS) still remained disappointing with only 3.1 and 2.0 mo, respectively[28]. In combination with TACE, sorafenib was able to induce a significant survival benefit compared to TACE only in patients with type B (13 mo *vs* 6 mo) or type C (15 mo *vs* 10 mo) in a stud enrolling 99 patients[29]. Similar results were obtained in combination with radiofrequency ablation (RFA)[30]. Still, prospective randomized controlled trials on sorafenib or regorafenib monotherapy in this setting are missing and the effect of the combination approach is probably overruling the currently available results[27].

Different combination studies of sorafenib and other agents have been performed. Interestingly, vitamin K was shown to enhance the antitumor effects of sorafenib via reduction of expression of des-γ-carboxy prothrombin (DCP), a proangiogenic growth factor that can also trigger signaling *via* c-Met and which is commonly upregulated after sorafenib treatment[31,32].

Combination studies with other targeted agents showed only a modest increase in survival but had significant increase in drug related adverse events or even showed a worse outcome than single agents like the sorafenib/erlotinib combination[33]. These agents have also been investigated in earlier disease stages but could not demonstrate a survival benefit in combination with locoregional approaches like TACE or in an adjuvant setting[7].

In summary, sorafenib and lenvatinib are options for first-line therapy of advanced HCC, while regorafenib, cabozantinib and ramucirumab can be used as second-line options afterwards.

**Immune checkpoint inhibitors and novel immunotherapy targets in HCC**

The development of immune checkpoint inhibitors like anti-CTLA4 (ipilimumab, tremelimumab) or anti-PD-1/PD-L1 (nivolumab, pembrolizumab, tislelizumab, camrelizumab, atezolizumab, durvalumab, avelumab) antibodies has dramatically changed clinical oncology nowadays and achieved sustained treatment responses in an unprecedented manner across different cancer types. Chronic inflammation due to the various underlying etiologies is a mainstay of HCC development. Different immune cell subtypes (T cells, macrophages, Kupffer cells) are currently intensively investigated to understand their role in HCC pathogenesis and to exploit them as novel and specific therapeutic approaches for this disease. Kupffer cells and CD8+ T cells in HCC have been shown to express high levels of PD-1 and PD-L1, thus playing a key role in the immune evasive phenotype of HCC. High expression of PD-L1 on tumor cells was associated with poorer outcome in HCC patients[34-37].

Several immune checkpoint inhibitors are currently investigated in clinical trials as single agents or in combination with neo-epitope releasing locoregional therapies, epigenetic drugs or conventional targeted agents[38].

In a phase 1 study, tremelimumab reached a clinical disease control rate of 76.4% with 17.6% of patients achieving partial response[39]. In combination with subtotal radiofrequency ablation or chemoablation, 26.3% of patients reached partial response and a median overall survival of 12.3 mo. Responders also showed increased infiltration of CD8+ T cells and HCV positive patients had a significant reduction in viral load[40].

Based on positive results of the phase 1/2 CheckMate-040 study, nivolumab received FDA approval as a 2nd line therapy option in HCC[41]. An objective response rate of 20% was reached overall, and patients expressing PD-L1 on tumor cells even reached 26%. A subgroup analysis revealed disease control rates of up to 75% and a median duration of response of 9.9 mo. Results of the phase 3 CheckMate-459 study that compares nivolumab vs sorafenib are still pending.

Similar results were obtained for pembrolizumab in the KEYNOTE-224 study. Here, 17% of patients had partial response, 44% had stable disease and overall 77% of responding patients had durable responses of 9 mo or more. Pembrolizumab also received FDA approval as a 2nd line therapy after sorafenib therapy.

Several further studies are currently ongoing to evaluate these checkpoint inhibitors in first or second line therapy of HCC, including the HIMALAYA study that explores durvalumab alone or in combination with tremelimumab *vs* sorafenib or the IMbrave50 study that combines atezolizumab with bevacizumab *vs* sorafenib[42].

Due to its physiologic role in clearing portal vein blood flow from potentially harmful gut content, the liver is a key immunologic organ and contains a high proportion of macrophages (Kupffer cells) and other cells of lymphoid lineage, including B and T cells as well as natural killer (NK) and NKT cells[43]. As outlined above, HCC commonly develops on the basis of chronic inflammatory liver injury and exploiting the immunologic repertoire of the liver. The first promising results of immune checkpoint inhibitors in HCC further support this approach. Different technologies have been developed to apply an adaptive cell transfer as a therapeutic option also in HCC, including application of tumor infiltrating lymphocytes, cytokine-induced killer cells or fostering neo-epitope release by locoregional ablation techniques[44-46].

Recently, chimeric antigen receptor-engineered T (CAR-T) cells yielded outstanding responses in hematologic malignancies and received FDA approval for the treatment of acute lymphoblastic leukemia[47,48] and diffuse large B-cell lymphoma[49,50]. In brief, T cells of patients are harvested, genetically modified, expanded and re-infused into the patient. CARs consist of an extracellular antigen recognition domain, a hinge/spacer domain, a transmembrane domain and a T cell activation domain (CD3). CARs of the 2nd and 3rd generation have additional costimulatory molecules like CD27 or CD134 in between the transmembrane and the CD3 domain to achieve prolonged T cell expansion and antitumor effects[51]. Success of CAR-T cells in solid tumors is limited by their broader mutational load compared to hematologic malignancies and suitable tumor antigens are thus more difficult to identify. An additional hurdle is the localization of modified T cells to the tumor, which is influenced by tumor angiogenesis, low levels of chemokines to attract T cells into the tumor and, esp. in HCC, a tumor microenvironment (stroma, fibrosis) that does not permit sufficient tissue penetration of large molecules[52]. The latter can be overcome by selective local administration like hepatic artery infusion which makes HCC an interesting option for this therapy approach and several studies using CAR-T cells were therefore initiated in HCC[44,53] (Table 3). Interestingly, most studies use the cell surface glycoprotein glypican-3 (GPC3) as an antigen, which is highly expressed in HCC but not in other adult tissues and has been linked to poor prognosis of HCC[54,55]. Preclinically, CAR-T cells targeting GPC3 were able to eliminate HCC cells and prolong survival of tumor-bearing mice[56,57]. Ongoing studies also evaluate different application routes like hepatic artery infusion, systemic infusion or combination with transarterial (chemo-)embolization and the effect of lymphodepleting conditioning. Preliminary results from study NCT02395250 indicate that GPC3-CAR-T cells are safe in relapsed or refractory Chinese HCC patients. In this study, 1 partial response and 2 stable diseases were observed as best response (from 6 evaluable patients), all durable for more than one year[58].

**Epigenetic therapy with HDAC inhibitors in HCC**

Epigenetic dysregulation of gene activity is essentially involved in HCC tumorigenesis as evidenced by dysregulation of histone deacetylases *in vitro*, *in vivo* and *in situ*[59,60]. Treatment with HDAC inhibitors (HDACi) therefore represents an attractive therapeutic option in liver cancer that addresses different molecular mechanisms compared to chemotherapy or targeted therapies to inhibit tumor cell growth and promote cell death. HDACi inhibitors commonly inhibit cell cycle progression by re-expression of p21cip1/waf1 in a p53-dependent manner but can also mediate alternative cell death pathways like unfolded protein response and ER stress pathways due to their non-specific acetylation of proteins also outside the nucleus[61-65]. Although some case reports showed a positive effect of HDACi in combination with sorafenib in HCC[66], most studies are disappointing so far. A phase 2 study using belinostat (inhibitor of all zinc dependent HDAC isoforms) in unresectable HCC showed a PFS and OS of 2.6 and 6.6 mo, respectively[67]. The SHELTER investigated resminostat (oral pan-HDACi with predominant activity against HDAC1, 2 and 3) in combination with sorafenib in a 2nd line setting of advanced HCC and demonstrated an OS of 8.0 mo, while monotherapy resminostat reached only 4.1 mo[68]. Interestingly, this combination was also used in a 1st line setting in Asian patients but did not provide evidence for an OS benefit over sorafenib[69].

These studies, like others investigating tyrosine kinase inhibitors, usually enroll patients with progressive, unresectable, locally advanced or metastatic HCC with an overall poor prognosis that limits the chance of achieving PFS or OS advantages[70]. Yet, epigenetic therapies may be able to overcome such hurdles and even enhance the results of immune checkpoint inhibitors in HCC. Epigenetic targeting with the enhancer of zeste homolog 2 (EZH2), a histone-lysine-N-methyltransferase, inhibitor 3-Deazaneplanocin A (DZNep) and the DNA methyltransferase 1 (DNMT1) inhibitor 5-azacytidine reactivated transcriptionally repressed chemokines genes and augmented T cell trafficking to the tumor[71]. Consequently, epigenetic pretreatment may lead to priming of so-called immune cold tumors also in HCC[71,72].

**Novel biomarkers to improve patient outcome**

HCC patients are commonly stratified based on their liver function capacity as assessed by Child-Pugh score, which overall seems to be a better predictor for treatment outcome than the underlying etiology of HCC[70,73]. Biomarkers that could predict treatment response are therefore urgently needed, esp. for targeted therapies and immunotherapy approaches[74].

In a recent biomarker analysis of the sorafenib phase 3 STORM trial (BIOSTORM), none of the biomarkers related to angiogenesis or cell proliferation or other molecular markers like gene signatures or mutations could predict a treatment benefit or recurrence-free survival (RFS). Only p-ERK and microvascular invasion were associated with poor RFS. This study proposed a new 146-gene signature that identified about 30% of patients which benefit from sorafenib treatment. Interestingly, those patients were also enriched in CD4+ T and B cells, NK cells and were associated with signature of poor response to immune checkpoint inhibitors[75].

Analysis of the regorafenib phase 3 study in HCC (RESORCE), also recently identified a plasma protein expression profile [angiopoietin 1, cystatin B, oxidized low density lipoprotein (LDL) receptor 1, latency associated peptide of transforming growth factor β (TGF-β) and macrophage inflammatory protein 1α (MIP1α)] and 9 plasma miRNAs that were associated with increased overall survival and time to progression. The proposed soluble plasma protein biomarkers are also known to play a role in inflammation and HCC pathogenesis. Interestingly, none of these predictive biomarkers was so far shown to have prognostic relevance[76].

Overall, the identification and validation of biomarkers in HCC was previously limited by the availability of tissue specimens as international guidelines did not mandate a biopsy sample for diagnostic purposes. Recently, guidelines from EASL recommend taking tissue and liquid biopsies from HCC patients participating in clinical studies which could improve this situation[77]. Biomarker analyses in HCC are often limited by small sample size in respective subgroup analyses due to the diverse etiologic backgrounds like viral hepatitis, NASH/NAFLD, cirrhosis status etc. which all significantly impact on the underlying chronic inflammation or have direct influence on oncogenic pathways.

Numerous new diagnostic and prognostic biomarkers like GPC-3 or c-Met have been proposed recently and were also translated into CAR-T cell based therapy approaches (see above) but no clear predictive biomarker for either targeted therapies or for immune checkpoint inhibitors is available so far[78,79].

Liquid biopsies are capable of detecting genetic and epigenetic alterations as well as expression patterns of DNA, RNA and miRNA from circulating tumor cells, cell-free nucleic acids or from exosomes. Success rate of these technologies is still variable depending on tumor size and stage but with further technological advances, e.g. on next generation sequencing from cell-free DNA, it is rapidly maturing to clinical applicability[80-82].

Assessment of metabolic pathways including proteomics and glycomics could further contribute to biomarker development although current approaches, e.g. detection of CD44v9[83] or Hippocalcin-like 1 (HPCAL1)[84], are used for diagnostic or prognostic settings or to predict disease recurrence and have not been linked to treatment responses.

**New preclinical models to improve HCC therapy**

The successful development of novel drugs is largely dependent on the availability of suitable and predictive preclinical models. Besides the specific biochemical (cell-free) inhibition of a distinct target, novel compounds need to prove their potency in various *in vitro* and *in vivo* model systems before entering human clinical trials.

In the past, high throughput screening was performed on 2D cultures using immortalized cell lines. Although this approach allowed screening large numbers of cell lines, it lacks the complex interaction of different cell types and matrix structures within real tissue. Consequently, more complex 3D culture systems were established, also from primary human cancer samples, that also contain components of extracellular matrix and additional cell types as fibroblasts[85,86]. Recently, spheroids and organoids that mimic organ structure and aggressiveness of human HCC have been established as tools for drug sensitivity screening[87,88]. To reflect the genetic heterogeneity of human cancers, mixtures of barcoded tumor cell lines can be subjected to high throughput screening. This technology secures homogeneous drug exposure to genetically different cell types at the same time and was shown to identify responders and non-responders to specific treatments as well as to create new biomarker hypotheses[89].

Precision-cut liver slices represent an interesting *ex vivo* model system for drug development. Complex tissue architecture is preserved and the model allows to investigate different pathophysiologic conditions and drug testing on primary human tissue samples[90,91].

Animal models, however, still represent standard models for early drug development approaches. As HCC usually develops on the background of chronic underlying liver diseases associated with chronic inflammation, viral infection or fibrotic remodeling, and clear oncogenic driver mutations have not been identified yet, finding suitable and appropriate models still remains an urgent task. An ideal model would therefore in parallel describe the underlying liver disease and tumor development. The increasing role of immunotherapies in HCC also urgently warrants the development of respective immunocompetent models (Table 4)[92].

The subcutaneous implantation of HCC cell lines was extensively used in the past and still has value when using primary cell lines or tissue explants (patient-derived xenograft models). These models are relatively easy to handle and provide an easy readout of tumor growth by caliper measurement. Orthotopic implantation reflects the primary site of tumor formation and crossplay with liver matrix and cells but requires surgical expertise and more advanced imaging technologies to monitor tumor growth. Both systems need immunodeficient mice unless syngeneic murine tumors are used. This limits the application of those models for studying immunotherapy approaches.

As human HCC can also develop upon chronic exposure to toxic agents, chemical induction in mice using diethylnitrosamine (DEN) has been established as a standard method but is limited by high variability of tumor formation and time to develop tumors[93]. Yet, chemical models can easily be combined with other approaches (*e.g.*, fibrosis induction, NASH models, alcohol) and thus provide an option for rapid evaluation of novel drugs under distinct pathophysiologic conditions[94-97].

Genetically engineered mice (GEM) are useful tools to study the contribution and effects of individual genes on HCC pathogenesis. They are technically more demanding and may have a longer latency period than other models. With the option of targeted knock-in and knock-out systems and combinations of those approaches, distinct molecular backgrounds can be analyzed. As today no clear single oncogenic driver for HCC development has been identified, several GEM models have been established as useful tools, *e.g.*, p53-deficient[98] mice or mice overexpressing MYC[99] or WNT pathway components[100,101]. The option to study HBV and HCV transgenic mice is of special interest. We have shown previously that dependent on the host genetic background, ER stress pathways can be activated that are known to lead to cellular stress and chronic inflammation and that is involved in fibrogenesis and ultimately also HCC pathogenesis[102,103].

As outlined above, HCC commonly develops on the basis of an underlying chronic liver disease. Therefore, specific liver disease models are of very high relevance to study HCC pathogenesis and explore new therapeutic options[92]. Several small animal models for the development of liver fibrosis and cirrhosis are currently established. Application of carbon tetrachloride (CCl4) or thioacetamide (in combination with ethanol)[104] leads to rapid fibrosis development and acute inflammation[105]. While easy to handle, these models can be combined with GEM or orthotopic transplantation models and provide a good tool to study tumor cells with a fibrotic microenvironment.

Although less clear in its pathogenesis for HCC, the increasing prevalence of metabolic liver diseases associated with diabetes mellitus, adipositas, hyperlipidemia, hypertriglyceridemia and metabolic syndrome puts models for NAFLD/NASH in the focus of today’s research models[106-108]. NAFLD and NASH can easily be induced by dietary models, *e.g.*, using a high-fat (HFD), high-cholesterol (HCD), high-fructose or methionine and choline-deficient (MCD) diets or combinations of those classical inducers[109]. Yet, dietary models are often limited by not completely following the human course of disease, *e.g.*, lacking HCC formation for high-fat diet or lacking obesity for the MCD diet. Therefore, models have been further refined by combining the dietary stimulus with distinct genetic models like PTEN-deficient mice, MC4R (melanocortin 4 receptor) or ALR (augmenter of liver regeneration) knockouts that reliably lead to HCC formation in 60% to 100% after approximately one year[110]. Recently, it was shown that HFD and HCD triggers liver cancer formation in an ApoE/LDL-receptor double knockout mouse, linking metabolic stress and atherosclerosis to HCC formation[111].

**Conclusion**

Integrative and comprehensive molecular and genomic analyses could classify hepatocellular carcinoma on the basis of landscapes of genetic and molecular signatures (Tables 1 and 2) which could then lead to the identification of predictive biomarkers for novel treatment options and then impact HCC trial design and patient outcome. Consequently, all HCCs should be biopsied and specimen should be intensively investigated applying “omics”-technologies for real precision medicine approaches. Furthermore, the biological roles of the identified driver genes in HCC must be analyzed in the deeper integration of inter- and intratumoral, interpatient, and inter-ethnic tumor heterogeneity in more detail. Deeper knowledge about those drivers is urgently needed, as the underlying pathogenesis of HCC is complex and currently shifting from chronic viral infections to more metabolically driven tumorigenesis as seen in NASH. As inflammation seems to be a common ground for HCC development, it is not surprising that immune checkpoint inhibitors are moving into first line therapy setting and it is expected that this compound class will also significantly shift the therapeutic landscape in HCC soon. Therefore, tumor models with complex genetically engineering are an essential drug development and technology transfer tool closing the gap between *in vitro* experiments and intensive clinical trials in future (Table 4). Finally, artificial intelligence and machine learning could essentially help to analyze, to classify and to interpret the dramatically increasing and high-dimensional amount of transcriptomic, genomic, epigenomic, metabolic, proteomic and imaging data in HCC[112-114]. The desirable aims of such approaches will be to (1) identify cancer drug targets, (2) predict anticancer sensitivity, toxicity and cancer resistance, and (3) give robust recommendations for therapeutic strategies in the future[115,116].

Overall, the better understanding of the molecular pathogenesis of HCC allows for more stringent patient selection criteria in biomarker-driven studies that can improve patient outcome.

**REFERENCES**

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Marengo A**, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. *Annu Rev Med* 2016; **67**: 103-117 [PMID: 26473416 DOI: 10.1146/annurev-med-090514-013832]

3 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 7-34 [PMID: 30620402 DOI: 10.3322/caac.21551]

4 **Khemlina G**, Ikeda S, Kurzrock R. The biology of Hepatocellular carcinoma: implications for genomic and immune therapies. *Mol Cancer* 2017; **16**: 149 [PMID: 28854942 DOI: 10.1186/s12943-017-0712-x]

5 **Zucman-Rossi J**, Villanueva A, Nault JC, Llovet JM. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. *Gastroenterology* 2015; **149**: 1226-1239.e4 [PMID: 26099527 DOI: 10.1053/j.gastro.2015.05.061]

6 **Gosalia AJ**, Martin P, Jones PD. Advances and Future Directions in the Treatment of Hepatocellular Carcinoma. *Gastroenterol Hepatol* (NY) 2017; **13**: 398-410 [PMID: 28867968]

7 **Ikeda K**. Recent advances in medical management of hepatocellular carcinoma. *Hepatol Res* 2019; **49**: 14-32 [PMID: 30308081 DOI: 10.1111/hepr.13259]

8 **Han B**, Li C, Meng H, Gomes Romeiro F, Mancuso A, Zhou Z, Levi Sandri GB, Xu Y, Han T, Han L, Shao L, Qi X. Efficacy and safety of external-beam radiation therapy for hepatocellular carcinoma: An overview of current evidence according to the different target population. *Biosci Trends* 2019; **13**: 10-22 [PMID: 30799321 DOI: 10.5582/bst.2018.01261]

9 **Qi X**, Zhao Y, Li H, Guo X, Han G. Management of hepatocellular carcinoma: an overview of major findings from meta-analyses. *Oncotarget* 2016; **7**: 34703-34751 [PMID: 27167195 DOI: 10.18632/oncotarget.9157]

10 **Eatrides J**, Wang E, Kothari N, Kim R. Role of Systemic Therapy and Future Directions for Hepatocellular Carcinoma. *Cancer Control* 2017; **24**: 1073274817729243 [PMID: 28975834 DOI: 10.1177/1073274817729243]

11 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

12 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]

13 **Ikeda K**, Kudo M, Kawazoe S, Osaki Y, Ikeda M, Okusaka T, Tamai T, Suzuki T, Hisai T, Hayato S, Okita K, Kumada H. Phase 2 study of lenvatinib in patients with advanced hepatocellular carcinoma. *J Gastroenterol* 2017; **52**: 512-519 [PMID: 27704266 DOI: 10.1007/s00535-016-1263-4]

14 **Kudo M**, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018; **391**: 1163-1173 [PMID: 29433850 DOI: 10.1016/S0140-6736(18)30207-1]

15 **Bruix J**, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G; RESORCE Investigators. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **389**: 56-66 [PMID: 27932229 DOI: 10.1016/S0140-6736(16)32453-9]

16 **Abou-Alfa GK**, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, Cicin I, Merle P, Chen Y, Park JW, Blanc JF, Bolondi L, Klümpen HJ, Chan SL, Zagonel V, Pressiani T, Ryu MH, Venook AP, Hessel C, Borgman-Hagey AE, Schwab G, Kelley RK. Cabozantinib in Patients with Advanced and Progressing Hepatocellular Carcinoma. *N Engl J Med* 2018; **379**: 54-63 [PMID: 29972759 DOI: 10.1056/NEJMoa1717002]

17 **Zhu AX**, Kang YK, Yen CJ, Finn RS, Galle PR, Llovet JM, Assenat E, Brandi G, Pracht M, Lim HY, Rau KM, Motomura K, Ohno I, Merle P, Daniele B, Shin DB, Gerken G, Borg C, Hiriart JB, Okusaka T, Morimoto M, Hsu Y, Abada PB, Kudo M; REACH-2 study investigators. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2019; **20**: 282-296 [PMID: 30665869 DOI: 10.1016/S1470-2045(18)30937-9]

18 **Han P**, Li H, Jiang X, Zhai B, Tan G, Zhao D, Qiao H, Liu B, Jiang H, Sun X. Dual inhibition of Akt and c-Met as a second-line therapy following acquired resistance to sorafenib in hepatocellular carcinoma cells. *Mol Oncol* 2017; **11**: 320-334 [PMID: 28164434 DOI: 10.1002/1878-0261.12039]

19 **Ueki T**, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. Expression of hepatocyte growth factor and its receptor, the c-met proto-oncogene, in hepatocellular carcinoma. *Hepatology* 1997; **25**: 619-623 [PMID: 9049208 DOI: 10.1002/hep.510250321]

20 **Kaposi-Novak P**, Lee JS, Gòmez-Quiroz L, Coulouarn C, Factor VM, Thorgeirsson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. *J Clin Invest* 2006; **116**: 1582-1595 [PMID: 16710476 DOI: 10.1172/JCI27236]

21 **García-Vilas JA**, Medina MÁ. Updates on the hepatocyte growth factor/c-Met axis in hepatocellular carcinoma and its therapeutic implications. *World J Gastroenterol* 2018; **24**: 3695-3708 [PMID: 30197476 DOI: 10.3748/wjg.v24.i33.3695]

22 **Bouattour M**, Raymond E, Qin S, Cheng AL, Stammberger U, Locatelli G, Faivre S. Recent developments of c-Met as a therapeutic target in hepatocellular carcinoma. *Hepatology* 2018; **67**: 1132-1149 [PMID: 28862760 DOI: 10.1002/hep.29496]

23 **Qi XS**, Guo XZ, Han GH, Li HY, Chen J. MET inhibitors for treatment of advanced hepatocellular carcinoma: A review. *World J Gastroenterol* 2015; **21**: 5445-5453 [PMID: 25987766 DOI: 10.3748/wjg.v21.i18.5445]

24 **Llovet JM**, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, Kang YK, Assenat E, Lim HY, Boige V, Mathurin P, Fartoux L, Lin DY, Bruix J, Poon RT, Sherman M, Blanc JF, Finn RS, Tak WY, Chao Y, Ezzeddine R, Liu D, Walters I, Park JW. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. *J Clin Oncol* 2013; **31**: 3509-3516 [PMID: 23980090 DOI: 10.1200/JCO.2012.47.3009]

25 **Johnson PJ**, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Aviña J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, Decaens T, Tak WY, Jeng LB, Liu D, Ezzeddine R, Walters I, Cheng AL. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J Clin Oncol* 2013; **31**: 3517-3524 [PMID: 23980084 DOI: 10.1200/JCO.2012.48.4410]

26 **Ogasawara S**, Chiba T, Ooka Y, Kanogawa N, Saito T, Motoyama T, Suzuki E, Tawada A, Kanai F, Yokosuka O. Sorafenib treatment in Child-Pugh A and B patients with advanced hepatocellular carcinoma: safety, efficacy and prognostic factors. *Invest New Drugs* 2015; **33**: 729-739 [PMID: 25861764 DOI: 10.1007/s10637-015-0237-3]

27 **Qi X**, Guo X. Sorafenib for the treatment of hepatocellular carcinoma with portal vein tumour thrombosis: a systematic review of comparative studies. *Prz Gastroenterol* 2015; **10**: 142-147 [PMID: 26516379 DOI: 10.5114/pg.2015.52470]

28 **Jeong SW**, Jang JY, Shim KY, Lee SH, Kim SG, Cha SW, Kim YS, Cho YD, Kim HS, Kim BS, Kim KH, Kim JH. Practical effect of sorafenib monotherapy on advanced hepatocellular carcinoma and portal vein tumor thrombosis. *Gut Liver* 2013; **7**: 696-703 [PMID: 24312711 DOI: 10.5009/gnl.2013.7.6.696]

29 **Zhu K**, Chen J, Lai L, Meng X, Zhou B, Huang W, Cai M, Shan H. Hepatocellular carcinoma with portal vein tumor thrombus: treatment with transarterial chemoembolization combined with sorafenib--a retrospective controlled study. *Radiology* 2014; **272**: 284-293 [PMID: 24708192 DOI: 10.1148/radiol.14131946]

30 **Giorgio A**, Merola MG, Montesarchio L, Merola F, Santoro B, Coppola C, Gatti P, Amendola F, DI Sarno A, Calvanese A, Matteucci P, Giorgio V. Sorafenib Combined with Radio-frequency Ablation Compared with Sorafenib Alone in Treatment of Hepatocellular Carcinoma Invading Portal Vein: A Western Randomized Controlled Trial. *Anticancer Res* 2016; **36**: 6179-6183 [PMID: 27793949 DOI: 10.21873/anticanres.11211]

31 **Jung DH**, Hwang S, Song GW, Ryoo BY, Kim N, Tak E, Hong HN. An interim safety analysis of hepatocellular carcinoma patients administrating oral vitamin K with or without sorafenib. *Korean J Hepatobiliary Pancreat Surg* 2015; **19**: 1-5 [PMID: 26155269 DOI: 10.14701/kjhbps.2015.19.1.1]

32 **Haruna Y**, Hasegawa N, Imanaka K, Kawamoto S, Inoue A. Clinical Impact of Vitamin K Dosing on Sorafenib Treatment for Hepatocellular Carcinoma. *J Cancer* 2017; **8**: 1988-1994 [PMID: 28819398 DOI: 10.7150/jca.18900]

33 **Zhu AX**, Rosmorduc O, Evans TR, Ross PJ, Santoro A, Carrilho FJ, Bruix J, Qin S, Thuluvath PJ, Llovet JM, Leberre MA, Jensen M, Meinhardt G, Kang YK. SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2015; **33**: 559-566 [PMID: 25547503 DOI: 10.1200/JCO.2013.53.7746]

34 **Wu K**, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res* 2009; **69**: 8067-8075 [PMID: 19826049 DOI: 10.1158/0008-5472.CAN-09-0901]

35 **Calderaro J**, Rousseau B, Amaddeo G, Mercey M, Charpy C, Costentin C, Luciani A, Zafrani ES, Laurent A, Azoulay D, Lafdil F, Pawlotsky JM. Programmed death ligand 1 expression in hepatocellular carcinoma: Relationship With clinical and pathological features. *Hepatology* 2016; **64**: 2038-2046 [PMID: 27359084 DOI: 10.1002/hep.28710]

36 **Long J**, Qu T, Pan XF, Tang X, Wan HH, Qiu P, Xu YH. Expression of programmed death ligand-1 and programmed death 1 in hepatocellular carcinoma and its clinical significance. *J Cancer Res Ther* 2018; **14**: S1188-S1192 [PMID: 30539869 DOI: 10.4103/0973-1482.204850]

37 **Dai X**, Xue J, Hu J, Yang SL, Chen GG, Lai PBS, Yu C, Zeng C, Fang X, Pan X, Zhang T. Positive Expression of Programmed Death Ligand 1 in Peritumoral Liver Tissue is Associated with Poor Survival after Curative Resection of Hepatocellular Carcinoma. *Transl Oncol* 2017; **10**: 511-517 [PMID: 28558264 DOI: 10.1016/j.tranon.2017.03.009]

38 **Xu F**, Jin T, Zhu Y, Dai C. Immune checkpoint therapy in liver cancer. *J Exp Clin Cancer Res* 2018; **37**: 110 [PMID: 29843754 DOI: 10.1186/s13046-018-0777-4]

39 **Sangro B**, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, Barrera P, Riezu-Boj JI, Larrea E, Alfaro C, Sarobe P, Lasarte JJ, Pérez-Gracia JL, Melero I, Prieto J. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013; **59**: 81-88 [PMID: 23466307 DOI: 10.1016/j.jhep.2013.02.022]

40 **Duffy AG**, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, Pratt D, Davis JL, Hughes MS, Heller T, ElGindi M, Uppala A, Korangy F, Kleiner DE, Figg WD, Venzon D, Steinberg SM, Venkatesan AM, Krishnasamy V, Abi-Jaoudeh N, Levy E, Wood BJ, Greten TF. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017; **66**: 545-551 [PMID: 27816492 DOI: 10.1016/j.jhep.2016.10.029]

41 **El-Khoueiry AB**, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]

42 **Okusaka T**, Ikeda M. Immunotherapy for hepatocellular carcinoma: current status and future perspectives. *ESMO Open* 2018; **3**: e000455 [PMID: 30622744 DOI: 10.1136/esmoopen-2018-000455]

43 **Bogdanos DP**, Gao B, Gershwin ME. Liver immunology. *Compr Physiol* 2013; **3**: 567-598 [PMID: 23720323 DOI: 10.1002/cphy.c120011]

44 **Zhang R**, Zhang Z, Liu Z, Wei D, Wu X, Bian H, Chen Z. Adoptive cell transfer therapy for hepatocellular carcinoma. *Front Med* 2019; **13**: 3-11 [PMID: 30659408 DOI: 10.1007/s11684-019-0684-x]

45 **Wissniowski TT**, Hänsler J, Neureiter D, Frieser M, Schaber S, Esslinger B, Voll R, Strobel D, Hahn EG, Schuppan D. Activation of tumor-specific T lymphocytes by radio-frequency ablation of the VX2 hepatoma in rabbits. *Cancer Res* 2003; **63**: 6496-6500 [PMID: 14559842]

46 **Behm B**, Di Fazio P, Michl P, Neureiter D, Kemmerling R, Hahn EG, Strobel D, Gress T, Schuppan D, Wissniowski TT. Additive antitumour response to the rabbit VX2 hepatoma by combined radio frequency ablation and toll like receptor 9 stimulation. *Gut* 2016; **65**: 134-143 [PMID: 25524262 DOI: 10.1136/gutjnl-2014-308286]

47 **Park JH**, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, Sauter C, Wang Y, Santomasso B, Mead E, Roshal M, Maslak P, Davila M, Brentjens RJ, Sadelain M. Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. *N Engl J Med* 2018; **378**: 449-459 [PMID: 29385376 DOI: 10.1056/NEJMoa1709919]

48 **Maude SL**, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, Bader P, Verneris MR, Stefanski HE, Myers GD, Qayed M, De Moerloose B, Hiramatsu H, Schlis K, Davis KL, Martin PL, Nemecek ER, Yanik GA, Peters C, Baruchel A, Boissel N, Mechinaud F, Balduzzi A, Krueger J, June CH, Levine BL, Wood P, Taran T, Leung M, Mueller KT, Zhang Y, Sen K, Lebwohl D, Pulsipher MA, Grupp SA. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. *N Engl J Med* 2018; **378**: 439-448 [PMID: 29385370 DOI: 10.1056/NEJMoa1709866]

49 **Locke FL**, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, Lin Y, Braunschweig I, Hill BT, Timmerman JM, Deol A, Reagan PM, Stiff P, Flinn IW, Farooq U, Goy A, McSweeney PA, Munoz J, Siddiqi T, Chavez JC, Herrera AF, Bartlett NL, Wiezorek JS, Navale L, Xue A, Jiang Y, Bot A, Rossi JM, Kim JJ, Go WY, Neelapu SS. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncol* 2019; **20**: 31-42 [PMID: 30518502 DOI: 10.1016/S1470-2045(18)30864-7]

50 **Neelapu SS**, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, Timmerman JM, Stiff PJ, Friedberg JW, Flinn IW, Goy A, Hill BT, Smith MR, Deol A, Farooq U, McSweeney P, Munoz J, Avivi I, Castro JE, Westin JR, Chavez JC, Ghobadi A, Komanduri KV, Levy R, Jacobsen ED, Witzig TE, Reagan P, Bot A, Rossi J, Navale L, Jiang Y, Aycock J, Elias M, Chang D, Wiezorek J, Go WY. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med* 2017; **377**: 2531-2544 [PMID: 29226797 DOI: 10.1056/NEJMoa1707447]

51 **Spear TT**, Nagato K, Nishimura MI. Strategies to genetically engineer T cells for cancer immunotherapy. *Cancer Immunol Immunother* 2016; **65**: 631-649 [PMID: 27138532 DOI: 10.1007/s00262-016-1842-5]

52 **Long KB**, Young RM, Boesteanu AC, Davis MM, Melenhorst JJ, Lacey SF, DeGaramo DA, Levine BL, Fraietta JA. CAR T Cell Therapy of Non-hematopoietic Malignancies: Detours on the Road to Clinical Success. *Front Immunol* 2018; **9**: 2740 [PMID: 30559740 DOI: 10.3389/fimmu.2018.02740]

53 **Chen Y**, E CY, Gong ZW, Liu S, Wang ZX, Yang YS, Zhang XW. Chimeric antigen receptor-engineered T-cell therapy for liver cancer. *Hepatobiliary Pancreat Dis Int* 2018; **17**: 301-309 [PMID: 29861325 DOI: 10.1016/j.hbpd.2018.05.005]

54 **Haruyama Y**, Kataoka H. Glypican-3 is a prognostic factor and an immunotherapeutic target in hepatocellular carcinoma. *World J Gastroenterol* 2016; **22**: 275-283 [PMID: 26755876 DOI: 10.3748/wjg.v22.i1.275]

55 **Kaseb AO**, Hassan M, Lacin S, Abdel-Wahab R, Amin HM, Shalaby A, Wolff RA, Yao J, Rashid A, Vennapusa B, Feng J, Ohtomo T. Evaluating clinical and prognostic implications of Glypican-3 in hepatocellular carcinoma. *Oncotarget* 2016; **7**: 69916-69926 [PMID: 27655712 DOI: 10.18632/oncotarget.12066]

56 **Gao H**, Li K, Tu H, Pan X, Jiang H, Shi B, Kong J, Wang H, Yang S, Gu J, Li Z. Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. *Clin Cancer Res* 2014; **20**: 6418-6428 [PMID: 25320357 DOI: 10.1158/1078-0432.CCR-14-1170]

57 **Dargel C**, Bassani-Sternberg M, Hasreiter J, Zani F, Bockmann JH, Thiele F, Bohne F, Wisskirchen K, Wilde S, Sprinzl MF, Schendel DJ, Krackhardt AM, Uckert W, Wohlleber D, Schiemann M, Stemmer K, Heikenwälder M, Busch DH, Richter G, Mann M, Protzer U. T Cells Engineered to Express a T-Cell Receptor Specific for Glypican-3 to Recognize and Kill Hepatoma Cells In Vitro and in Mice. *Gastroenterology* 2015; **149**: 1042-1052 [PMID: 26052074 DOI: 10.1053/j.gastro.2015.05.055]

58 **Zhai B**, Shi D, Gao H, Qi X, Jiang H, Zhang Y, Chi J, Ruan H, Wang H, Ru QC, Li Z. A phase I study of anti-GPC3 chimeric antigen receptor modified T cells (GPC3 CAR-T) in Chinese patients with refractory or relapsed GPC3+ hepatocellular carcinoma (r/r GPC3+ HCC). *J Clin Oncol* 2017; **35**: 3049 [DOI: 10.1200/JCO.2017.35.15\_suppl.3049]

59 **Zhao J**, Gray SG, Greene CM, Lawless MW. Unmasking the pathological and therapeutic potential of histone deacetylases for liver cancer. *Expert Rev Gastroenterol Hepatol* 2019; **13**: 247-256 [PMID: 30791763 DOI: 10.1080/17474124.2019.1568870]

60 **Quint K**, Agaimy A, Di Fazio P, Montalbano R, Steindorf C, Jung R, Hellerbrand C, Hartmann A, Sitter H, Neureiter D, Ocker M. Clinical significance of histone deacetylases 1, 2, 3, and 7: HDAC2 is an independent predictor of survival in HCC. *Virchows Arch* 2011; **459**: 129-139 [PMID: 21713366 DOI: 10.1007/s00428-011-1103-0]

61 **Ocker M**, Schneider-Stock R. Histone deacetylase inhibitors: signalling towards p21cip1/waf1. *Int J Biochem Cell Biol* 2007; **39**: 1367-1374 [PMID: 17412634 DOI: 10.1016/j.biocel.2007.03.001]

62 **Di Fazio P**, Schneider-Stock R, Neureiter D, Okamoto K, Wissniowski T, Gahr S, Quint K, Meissnitzer M, Alinger B, Montalbano R, Sass G, Hohenstein B, Hahn EG, Ocker M. The pan-deacetylase inhibitor panobinostat inhibits growth of hepatocellular carcinoma models by alternative pathways of apoptosis. *Cell Oncol* 2010; **32**: 285-300 [PMID: 20208142 DOI: 10.3233/CLO-2010-0511]

63 **Ocker M**. Deacetylase inhibitors - focus on non-histone targets and effects. *World J Biol Chem* 2010; **1**: 55-61 [PMID: 21540990 DOI: 10.4331/wjbc.v1.i5.55]

64 **Montalbano R**, Waldegger P, Quint K, Jabari S, Neureiter D, Illig R, Ocker M, Di Fazio P. Endoplasmic reticulum stress plays a pivotal role in cell death mediated by the pan-deacetylase inhibitor panobinostat in human hepatocellular cancer cells. *Transl Oncol* 2013; **6**: 143-157 [PMID: 23544167 DOI: 10.1593/tlo.12271]

65 **Di Fazio P**, Waldegger P, Jabari S, Lingelbach S, Montalbano R, Ocker M, Slater EP, Bartsch DK, Illig R, Neureiter D, Wissniowski TT. Autophagy-related cell death by pan-histone deacetylase inhibition in liver cancer. *Oncotarget* 2016; **7**: 28998-29010 [PMID: 27058414 DOI: 10.18632/oncotarget.8585]

66 **Gahr S**, Wissniowski T, Zopf S, Strobel D, Pustowka A, Ocker M. Combination of the deacetylase inhibitor panobinostat and the multi-kinase inhibitor sorafenib for the treatment of metastatic hepatocellular carcinoma - review of the underlying molecular mechanisms and first case report. *J Cancer* 2012; **3**: 158-165 [PMID: 22514558 DOI: 10.7150/jca.4211]

67 **Yeo W**, Chung HC, Chan SL, Wang LZ, Lim R, Picus J, Boyer M, Mo FK, Koh J, Rha SY, Hui EP, Jeung HC, Roh JK, Yu SC, To KF, Tao Q, Ma BB, Chan AW, Tong JH, Erlichman C, Chan AT, Goh BC. Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. *J Clin Oncol* 2012; **30**: 3361-3367 [PMID: 22915658 DOI: 10.1200/JCO.2011.41.2395]

68 **Bitzer M**, Horger M, Giannini EG, Ganten TM, Wörns MA, Siveke JT, Dollinger MM, Gerken G, Scheulen ME, Wege H, Zagonel V, Cillo U, Trevisani F, Santoro A, Montesarchio V, Malek NP, Holzapfel J, Herz T, Ammendola AS, Pegoraro S, Hauns B, Mais A, Lauer UM, Henning SW, Hentsch B. Resminostat plus sorafenib as second-line therapy of advanced hepatocellular carcinoma - The SHELTER study. *J Hepatol* 2016; **65**: 280-288 [PMID: 26952006 DOI: 10.1016/j.jhep.2016.02.043]

69 **Tak WY**, Ryoo BY, Lim HY, Kim DY, Okusaka T, Ikeda M, Hidaka H, Yeon JE, Mizukoshi E, Morimoto M, Lee MA, Yasui K, Kawaguchi Y, Heo J, Morita S, Kim TY, Furuse J, Katayama K, Aramaki T, Hara R, Kimura T, Nakamura O, Kudo M. Phase I/II study of first-line combination therapy with sorafenib plus resminostat, an oral HDAC inhibitor, versus sorafenib monotherapy for advanced hepatocellular carcinoma in east Asian patients. *Invest New Drugs* 2018; **36**: 1072-1084 [PMID: 30198057 DOI: 10.1007/s10637-018-0658-x]

70 **Llovet JM**, Hernandez-Gea V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. *Clin Cancer Res* 2014; **20**: 2072-2079 [PMID: 24589894 DOI: 10.1158/1078-0432.CCR-13-0547]

71 **Hong YK**, Li Y, Pandit H, Li S, Pulliam Z, Zheng Q, Yu Y, Martin RCG. Epigenetic modulation enhances immunotherapy for hepatocellular carcinoma. *Cell Immunol* 2019; **336**: 66-74 [PMID: 30626493 DOI: 10.1016/j.cellimm.2018.12.010]

72 **Llopiz D**, Ruiz M, Villanueva L, Iglesias T, Silva L, Egea J, Lasarte JJ, Pivette P, Trochon-Joseph V, Vasseur B, Dixon G, Sangro B, Sarobe P. Enhanced anti-tumor efficacy of checkpoint inhibitors in combination with the histone deacetylase inhibitor Belinostat in a murine hepatocellular carcinoma model. *Cancer Immunol Immunother* 2019; **68**: 379-393 [PMID: 30547218 DOI: 10.1007/s00262-018-2283-0]

73 **Bruix J**, Cheng AL, Meinhardt G, Nakajima K, De Sanctis Y, Llovet J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: Analysis of two phase III studies. *J Hepatol* 2017; **67**: 999-1008 [PMID: 28687477 DOI: 10.1016/j.jhep.2017.06.026]

74 **Ocker M**. Biomarkers for hepatocellular carcinoma: What's new on the horizon? *World J Gastroenterol* 2018; **24**: 3974-3979 [PMID: 30254402 DOI: 10.3748/wjg.v24.i35.3974]

75 **Pinyol R**, Montal R, Bassaganyas L, Sia D, Takayama T, Chau GY, Mazzaferro V, Roayaie S, Lee HC, Kokudo N, Zhang Z, Torrecilla S, Moeini A, Rodriguez-Carunchio L, Gane E, Verslype C, Croitoru AE, Cillo U, de la Mata M, Lupo L, Strasser S, Park JW, Camps J, Solé M, Thung SN, Villanueva A, Pena C, Meinhardt G, Bruix J, Llovet JM. Molecular predictors of prevention of recurrence in HCC with sorafenib as adjuvant treatment and prognostic factors in the phase 3 STORM trial. *Gut* 2018; : [PMID: 30108162 DOI: 10.1136/gutjnl-2018-316408]

76 **Teufel M**, Seidel H, Köchert K, Meinhardt G, Finn RS, Llovet JM, Bruix J. Biomarkers Associated With Response to Regorafenib in Patients With Hepatocellular Carcinoma. *Gastroenterology* 2019; **156**: 1731-1741 [PMID: 30738047 DOI: 10.1053/j.gastro.2019.01.261]

77 **European Association for the Study of the Liver**; European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]

78 **Black AP**, Mehta AS. The search for biomarkers of hepatocellular carcinoma and the impact on patient outcome. *Curr Opin Pharmacol* 2018; **41**: 74-78 [PMID: 29772420 DOI: 10.1016/j.coph.2018.04.002]

79 **Lou J**, Zhang L, Lv S, Zhang C, Jiang S. Biomarkers for Hepatocellular Carcinoma. *Biomark Cancer* 2017; **9**: 1-9 [PMID: 28469485 DOI: 10.1177/1179299X16684640]

80 **Ng CKY**, Di Costanzo GG, Terracciano LM, Piscuoglio S. Circulating Cell-Free DNA in Hepatocellular Carcinoma: Current Insights and Outlook. *Front Med* (Lausanne) 2018; **5**: 78 [PMID: 29632864 DOI: 10.3389/fmed.2018.00078]

81 **Clark TA**, Chung JH, Kennedy M, Hughes JD, Chennagiri N, Lieber DS, Fendler B, Young L, Zhao M, Coyne M, Breese V, Young G, Donahue A, Pavlick D, Tsiros A, Brennan T, Zhong S, Mughal T, Bailey M, He J, Roels S, Frampton GM, Spoerke JM, Gendreau S, Lackner M, Schleifman E, Peters E, Ross JS, Ali SM, Miller VA, Gregg JP, Stephens PJ, Welsh A, Otto GA, Lipson D. Analytical Validation of a Hybrid Capture-Based Next-Generation Sequencing Clinical Assay for Genomic Profiling of Cell-Free Circulating Tumor DNA. *J Mol Diagn* 2018; **20**: 686-702 [PMID: 29936259 DOI: 10.1016/j.jmoldx.2018.05.004]

82 **Plagnol V**, Woodhouse S, Howarth K, Lensing S, Smith M, Epstein M, Madi M, Smalley S, Leroy C, Hinton J, de Kievit F, Musgrave-Brown E, Herd C, Baker-Neblett K, Brennan W, Dimitrov P, Campbell N, Morris C, Rosenfeld N, Clark J, Gale D, Platt J, Calaway J, Jones G, Forshew T. Analytical validation of a next generation sequencing liquid biopsy assay for high sensitivity broad molecular profiling. *PLoS One* 2018; **13**: e0193802 [PMID: 29543828 DOI: 10.1371/journal.pone.0193802]

83 **Kakehashi A**, Ishii N, Sugihara E, Gi M, Saya H, Wanibuchi H. CD44 variant 9 is a potential biomarker of tumor initiating cells predicting survival outcome in hepatitis C virus-positive patients with resected hepatocellular carcinoma. *Cancer Sci* 2016; **107**: 609-618 [PMID: 26882440 DOI: 10.1111/cas.12908]

84 **Zhang Y**, Liu Y, Duan J, Yan H, Zhang J, Zhang H, Fan Q, Luo F, Yan G, Qiao K, Liu J. Hippocalcin-like 1 suppresses hepatocellular carcinoma progression by promoting p21(Waf/Cip1) stabilization by activating the ERK1/2-MAPK pathway. *Hepatology* 2016; **63**: 880-897 [PMID: 26659654 DOI: 10.1002/hep.28395]

85 **Das V**, Bruzzese F, Konečný P, Iannelli F, Budillon A, Hajdúch M. Pathophysiologically relevant in vitro tumor models for drug screening. *Drug Discov Today* 2015; **20**: 848-855 [PMID: 25908576 DOI: 10.1016/j.drudis.2015.04.004]

86 **Sant S**, Johnston PA. The production of 3D tumor spheroids for cancer drug discovery. *Drug Discov Today Technol* 2017; **23**: 27-36 [PMID: 28647083 DOI: 10.1016/j.ddtec.2017.03.002]

87 **Khawar IA**, Park JK, Jung ES, Lee MA, Chang S, Kuh HJ. Three Dimensional Mixed-Cell Spheroids Mimic Stroma-Mediated Chemoresistance and Invasive Migration in hepatocellular carcinoma. *Neoplasia* 2018; **20**: 800-812 [PMID: 29981501 DOI: 10.1016/j.neo.2018.05.008]

88 **Jung HR**, Kang HM, Ryu JW, Kim DS, Noh KH, Kim ES, Lee HJ, Chung KS, Cho HS, Kim NS, Im DS, Lim JH, Jung CR. Cell Spheroids with Enhanced Aggressiveness to Mimic Human Liver Cancer In Vitro and In Vivo. *Sci Rep* 2017; **7**: 10499 [PMID: 28874716 DOI: 10.1038/s41598-017-10828-7]

89 **Yu C**, Mannan AM, Yvone GM, Ross KN, Zhang YL, Marton MA, Taylor BR, Crenshaw A, Gould JZ, Tamayo P, Weir BA, Tsherniak A, Wong B, Garraway LA, Shamji AF, Palmer MA, Foley MA, Winckler W, Schreiber SL, Kung AL, Golub TR. High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines. *Nat Biotechnol* 2016; **34**: 419-423 [PMID: 26928769 DOI: 10.1038/nbt.3460]

90 **Palma E**, Doornebal EJ, Chokshi S. Precision-cut liver slices: a versatile tool to advance liver research. *Hepatol Int* 2019; **13**: 51-57 [PMID: 30515676 DOI: 10.1007/s12072-018-9913-7]

91 **Kern MA**, Haugg AM, Eiteneuer E, Konze E, Drebber U, Dienes HP, Breuhahn K, Schirmacher P, Kasper HU. Ex vivo analysis of antineoplastic agents in precision-cut tissue slices of human origin: effects of cyclooxygenase-2 inhibition in hepatocellular carcinoma. *Liver Int* 2006; **26**: 604-612 [PMID: 16762006 DOI: 10.1111/j.1478-3231.2006.01268.x]

92 **Brown ZJ**, Heinrich B, Greten TF. Mouse models of hepatocellular carcinoma: an overview and highlights for immunotherapy research. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 536-554 [PMID: 29904153 DOI: 10.1038/s41575-018-0033-6]

93 **Uehara T**, Pogribny IP, Rusyn I. The DEN and CCl4 -Induced Mouse Model of Fibrosis and Inflammation-Associated Hepatocellular Carcinoma. *Curr Protoc Pharmacol* 2014; **66**: 14.30.1-14.3010 [PMID: 25181010 DOI: 10.1002/0471141755.ph1430s66]

94 **Arboatti AS**, Lambertucci F, Sedlmeier MG, Pisani G, Monti J, Álvarez ML, Francés DEA, Ronco MT, Carnovale CE. Diethylnitrosamine enhances hepatic tumorigenic pathways in mice fed with high fat diet (Hfd). *Chem Biol Interact* 2019; **303**: 70-78 [PMID: 30826251 DOI: 10.1016/j.cbi.2019.02.024]

95 **Henderson JM**, Polak N, Chen J, Roediger B, Weninger W, Kench JG, McCaughan GW, Zhang HE, Gorrell MD. Multiple liver insults synergize to accelerate experimental hepatocellular carcinoma. *Sci Rep* 2018; **8**: 10283 [PMID: 29980757 DOI: 10.1038/s41598-018-28486-8]

96 **Arboatti AS**, Lambertucci F, Sedlmeier MG, Pisani G, Monti J, Álvarez ML, Francés DEA, Ronco MT, Carnovale CE. Diethylnitrosamine Increases Proliferation in Early Stages of Hepatic Carcinogenesis in Insulin-Treated Type 1 Diabetic Mice. *Biomed Res Int* 2018; **2018**: 9472939 [PMID: 29850590 DOI: 10.1155/2018/9472939]

97 **Sun Q**, Long Z, Wu H, Liu Y, Wang L, Zhang X, Wang X, Hai C. Effect of alcohol on diethylnitrosamine-induced hepatic toxicity: Critical role of ROS, lipid accumulation, and mitochondrial dysfunction. *Exp Toxicol Pathol* 2015; **67**: 491-498 [PMID: 26198575 DOI: 10.1016/j.etp.2015.07.003]

98 **Katz SF**, Lechel A, Obenauf AC, Begus-Nahrmann Y, Kraus JM, Hoffmann EM, Duda J, Eshraghi P, Hartmann D, Liss B, Schirmacher P, Kestler HA, Speicher MR, Rudolph KL. Disruption of Trp53 in livers of mice induces formation of carcinomas with bilineal differentiation. *Gastroenterology* 2012; **142**: 1229-1239.e3 [PMID: 22342966 DOI: 10.1053/j.gastro.2012.02.009]

99 **Dolezal JM**, Wang H, Kulkarni S, Jackson L, Lu J, Ranganathan S, Goetzman ES, Bharathi SS, Beezhold K, Byersdorfer CA, Prochownik EV. Sequential adaptive changes in a c-Myc-driven model of hepatocellular carcinoma. *J Biol Chem* 2017; **292**: 10068-10086 [PMID: 28432125 DOI: 10.1074/jbc.M117.782052]

100 **Yim SY**, Shim JJ, Shin JH, Jeong YS, Kang SH, Kim SB, Eun YG, Lee DJ, Conner EA, Factor VM, Moore DD, Johnson RL, Thorgeirsson SS, Lee JS. Integrated Genomic Comparison of Mouse Models Reveals Their Clinical Resemblance to Human Liver Cancer. *Mol Cancer Res* 2018; **16**: 1713-1723 [PMID: 30082483 DOI: 10.1158/1541-7786.MCR-18-0313]

101 **Mokkapati S**, Niopek K, Huang L, Cunniff KJ, Ruteshouser EC, deCaestecker M, Finegold MJ, Huff V. β-catenin activation in a novel liver progenitor cell type is sufficient to cause hepatocellular carcinoma and hepatoblastoma. *Cancer Res* 2014; **74**: 4515-4525 [PMID: 24848510 DOI: 10.1158/0008-5472.CAN-13-3275]

102 **Churin Y**, Roderfeld M, Stiefel J, Würger T, Schröder D, Matono T, Mollenkopf HJ, Montalbano R, Pompaiah M, Reifenberg K, Zahner D, Ocker M, Gerlich W, Glebe D, Roeb E. Pathological impact of hepatitis B virus surface proteins on the liver is associated with the host genetic background. *PLoS One* 2014; **9**: e90608 [PMID: 24594856 DOI: 10.1371/journal.pone.0090608]

103 **Montalbano R**, Honrath B, Wissniowski TT, Elxnat M, Roth S, Ocker M, Quint K, Churin Y, Roederfeld M, Schroeder D, Glebe D, Roeb E, Di Fazio P. Exogenous hepatitis B virus envelope proteins induce endoplasmic reticulum stress: involvement of cannabinoid axis in liver cancer cells. *Oncotarget* 2016; **7**: 20312-20323 [PMID: 26967385 DOI: 10.18632/oncotarget.7950]

104 **Kornek M**, Raskopf E, Guetgemann I, Ocker M, Gerceker S, Gonzalez-Carmona MA, Rabe C, Sauerbruch T, Schmitz V. Combination of systemic thioacetamide (TAA) injections and ethanol feeding accelerates hepatic fibrosis in C3H/He mice and is associated with intrahepatic up regulation of MMP-2, VEGF and ICAM-1. *J Hepatol* 2006; **45**: 370-376 [PMID: 16780996 DOI: 10.1016/j.jhep.2006.03.017]

105 **Kim YO**, Popov Y, Schuppan D. Optimized Mouse Models for Liver Fibrosis. *Methods Mol Biol* 2017; **1559**: 279-296 [PMID: 28063051 DOI: 10.1007/978-1-4939-6786-5\_19]

106 **Hauff P**, Gottwald U, Ocker M. Early to Phase II drugs currently under investigation for the treatment of liver fibrosis. *Expert Opin Investig Drugs* 2015; **24**: 309-327 [PMID: 25547844 DOI: 10.1517/13543784.2015.997874]

107 **Santhekadur PK**, Kumar DP, Sanyal AJ. Preclinical models of non-alcoholic fatty liver disease. *J Hepatol* 2018; **68**: 230-237 [PMID: 29128391 DOI: 10.1016/j.jhep.2017.10.031]

108 **Charrez B**, Qiao L, Hebbard L. Hepatocellular carcinoma and non-alcoholic steatohepatitis: The state of play. *World J Gastroenterol* 2016; **22**: 2494-2502 [PMID: 26937137 DOI: 10.3748/wjg.v22.i8.2494]

109 **Lau JKC**, Zhang X, Yu J. Animal Models of Non-alcoholic Fatty Liver Diseases and Its Associated Liver Cancer. *Adv Exp Med Biol* 2018; **1061**: 139-147 [PMID: 29956212 DOI: 10.1007/978-981-10-8684-7\_11]

110 **Takakura K**, Oikawa T, Tomita Y, Mizuno Y, Nakano M, Saeki C, Torisu Y, Saruta M. Mouse models for investigating the underlying mechanisms of nonalcoholic steatohepatitis-derived hepatocellular carcinoma. *World J Gastroenterol* 2018; **24**: 1989-1994 [PMID: 29760542 DOI: 10.3748/wjg.v24.i18.1989]

111 **Kampschulte M**, Stöckl C, Langheinrich AC, Althöhn U, Bohle RM, Krombach GA, Stieger P, Churin Y, Kremer S, Dierkes C, Rath T, Roeb E, Roderfeld M. Western diet in ApoE-LDLR double-deficient mouse model of atherosclerosis leads to hepatic steatosis, fibrosis, and tumorigenesis. *Lab Invest* 2014; **94**: 1273-1282 [PMID: 25199052 DOI: 10.1038/labinvest.2014.112]

112 **Ma J**, Zhang L, Yang P, Zeng ZC, Xiang ZL. Integrated analysis of long noncoding RNA expression profiles in lymph node metastasis of hepatocellular carcinoma. *Gene* 2018; **676**: 47-55 [PMID: 29981417 DOI: 10.1016/j.gene.2018.07.001]

113 **Sang L**, Wang XM, Xu DY, Zhao WJ. Bioinformatics analysis of aberrantly methylated-differentially expressed genes and pathways in hepatocellular carcinoma. *World J Gastroenterol* 2018; **24**: 2605-2616 [PMID: 29962817 DOI: 10.3748/wjg.v24.i24.2605]

114 **Zhang L**, Huang Y, Ling J, Zhuo W, Yu Z, Shao M, Luo Y, Zhu Y. Screening and function analysis of hub genes and pathways in hepatocellular carcinoma via bioinformatics approaches. *Cancer Biomark* 2018; **22**: 511-521 [PMID: 29843214 DOI: 10.3233/CBM-171160]

115 **Huang S**, Cai N, Pacheco PP, Narrandes S, Wang Y, Xu W. Applications of Support Vector Machine (SVM) Learning in Cancer Genomics. *Cancer Genomics Proteomics* 2018; **15**: 41-51 [PMID: 29275361 DOI: 10.21873/cgp.20063]

116 **Chaudhary K**, Poirion OB, Lu L, Garmire LX. Deep Learning-Based Multi-Omics Integration Robustly Predicts Survival in Liver Cancer. *Clin Cancer Res* 2018; **24**: 1248-1259 [PMID: 28982688 DOI: 10.1158/1078-0432.CCR-17-0853]

117 **Cancer Genome Atlas Research Network**; Cancer Genome Atlas Research Network. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017; **169**: 1327-1341.e23 [PMID: 28622513 DOI: 10.1016/j.cell.2017.05.046]

118 **Chen J**, Zaidi S, Rao S, Chen JS, Phan L, Farci P, Su X, Shetty K, White J, Zamboni F, Wu X, Rashid A, Pattabiraman N, Mazumder R, Horvath A, Wu RC, Li S, Xiao C, Deng CX, Wheeler DA, Mishra B, Akbani R, Mishra L. Analysis of Genomes and Transcriptomes of Hepatocellular Carcinomas Identifies Mutations and Gene Expression Changes in the Transforming Growth Factor-β Pathway. *Gastroenterology* 2018; **154**: 195-210 [PMID: 28918914 DOI: 10.1053/j.gastro.2017.09.007]

119 **Désert R**, Nieto N, Musso O. Dimensions of hepatocellular carcinoma phenotypic diversity. *World J Gastroenterol* 2018; **24**: 4536-4547 [PMID: 30386103 DOI: 10.3748/wjg.v24.i40.4536]

120 **Lee JS**, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, Durnez A, Demetris AJ, Thorgeirsson SS. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 2004; **40**: 667-676 [PMID: 15349906 DOI: 10.1002/hep.20375]

121 **Boyault S**, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P, Zucman-Rossi J. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 2007; **45**: 42-52 [PMID: 17187432 DOI: 10.1002/hep.21467]

122 **Chiang DY**, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, LeBlanc AC, Donovan DJ, Thung SN, Solé M, Tovar V, Alsinet C, Ramos AH, Barretina J, Roayaie S, Schwartz M, Waxman S, Bruix J, Mazzaferro V, Ligon AH, Najfeld V, Friedman SL, Sellers WR, Meyerson M, Llovet JM. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. *Cancer Res* 2008; **68**: 6779-6788 [PMID: 18701503 DOI: 10.1158/0008-5472.CAN-08-0742]

123 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]

124 **Désert R**, Rohart F, Canal F, Sicard M, Desille M, Renaud S, Turlin B, Bellaud P, Perret C, Clément B, Lê Cao KA, Musso O. Human hepatocellular carcinomas with a periportal phenotype have the lowest potential for early recurrence after curative resection. *Hepatology* 2017; **66**: 1502-1518 [PMID: 28498607 DOI: 10.1002/hep.29254]

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**Table 1Known dysregulated pathways and genes in hepatocellular carcinoma with mode of action and frequency (modified from[4,5,92])**

|  |  |  |
| --- | --- | --- |
| **Pathways / genes** | **Alteration** | **Frequency in HCC** |
| AKT-mTOR-MAPK signaling | | |
| RPS6KA3  TSC1 and TSC2  PTEN  FGF3, FGF4 and FGF19  PI3KCA | Mutation  Mutation or deletion  Mutation or deletion  Amplification  Mutation | 2%%-9%  3%-8%  1%-3%  4%-6%  0%-2% |
| Angiogenesis | | |
| VEGFA | Amplification | 3%-7% |
| Antioxidation | | |
| NFE2L2  KEAP1 | Mutation  Mutation | 3%-6%  2%-8% |
| Cell cycle control/tumor suppressors | | |
| TP53\*  RB1  CCND1\* | Mutation or deletion  Mutation or deletion  Amplification | 12%-45%  3%-8%  5%-14% |
| Epigenetic and chromatin remodeling | | |
| ARID1A\*  ARID2\*  BAP1 | Mutation or deletion  Mutation  Mutation | 4%-17%  3%-18%  5%[117] |
| Immortalization/Telomere maintenance | | |
| ERT\* | Promotor mutation  Amplification | 54%-60%  5%-6% |
| JAK/STAT | | |
| JAK1 | Mutation | 5% |
| Metabolic pathways | | |
| Afamin  Apoptogenic protein 1, mitochondrial | Mutation | Up to 10%[117] |
| Oncogenes | | |
| MET\*  MYC | Amplification  Amplification | 30%-50%  4% |
| TGFβ pathway | | |
| Osteopontin  G2/mitotic- specific cyclin-B2  Cyclin- dependent kinase 1  Lymphoid enhancer- binding factor 1  Integrin α2 | Mutation | Up to 40%[118] |
| Wnt pathway | | |
| Catenin β1\*  AXIN1\* | Mutation  Mutation or deletion | 11%-37%  5%-15% |

HCC: Hepatocellular carcinoma.

**Table 2 Summary of classification schemes of hepatocellular carcinoma (modified from[119])**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **First author** | **Lee *et al*[120]** | **Boyault *et al*[121]** | **Chiang *et al*[122]** | **Hoshida *et al*[123]** | **Désert *et al*[124]** | **TCGA network[117]** |
| Year | 2004 | 2006 | 2008 | 2009 | 2017 | 2017 |
| HCC cases | 91 | 56 | 91 | 232 | 1133 | 559 |
| Number of subgroups | 2 | 6 | 5 | 3 | 4 | 3 |
| Names of classes | Cluster A/B | G1-G6 | CTNNB1-proliferation | S1-S3 | PP, PV, ECM, STEM | iCluster1-iCluster3 |
| Major applied technology for molecular profiling | | | | | | |
| Transcriptomics | X | X | X | X | X | X |
| Genetic Mutations |  | X |  |  |  | X |
| Copy number alterations |  |  | X |  |  | X |
| Metabolomics |  |  |  |  | X |  |
| Epigenomics |  | X (CDH1 and CDKN2A) |  |  |  | X |
| Proteomics |  |  |  |  |  | X |
| Major HCC Classes with clinic-pathological features and high mutation rates | | | | | | |
| Proliferative phenotype  Poor outcome  High AFP  Moderate to poor differentiation  P53 | A | G1, G2, G3 | Proliferation | S1+S2 | ECM+STEM | iCluster 1+3 |
| Non-proliferative phenotype  Good to moderate outcome  Low AFP  CTNNB1 | B | G5, G6 | CTNNB1 | S3 | PP+PV | iCluster 2 |

ECM: Extracellular matrix; PP: Periportal; PV: Perivenous; STEM: Stem/progenitor cells; HCC: Hepatocellular carcinoma.

**Table 3 Clinical trials with chimeric antigen receptor T cells cells in hepatocellular carcinoma**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **NCT** | **Antigen** | **Phase** | **Patients** | **Sponsor** | **Status** | **Comments** |
| NCT02715362 | GPC3 | I/II | 30 | Company | Recruiting | HAI |
| NCT03672305 | c-Met/PD-L1 | I | 50 | Academic | Not yet recruiting | IV |
| NCT02723942 | GPC3 | I/II | 60 | Academic | Completed |  |
| NCT03198546 | GPC3 | I | 30 | Academic | Recruiting |  |
| NCT02395250 | GPC3 | I | 13 | Academic | Completed | [58] |
| NCT03349255 | AFP | I | 18 | Company | Recruiting | IV *vs* HAI |
| NCT03130712 | GPC3 | I/II | 10 | Company | Recruiting | IT |
| NCT03084380 | GPC3 | I/II | 20 | Academic | Not yet recruiting | Combination with TACE |
| NCT029051881 | GPC3 | I | 14 | Academic | Not yet recruiting |  |
| NCT03302403 | GPC3 | I | 48 | Academic | Not yet recruiting |  |
| NCT03146234 | GPC3 | I | 20 | Academic | Recruiting |  |
| NCT01935843 | Her2 | I/II | 10 | Academic | Unknown |  |
| NCT02959151 | GPC3 | I/II | 20 | Company | Unknown |  |
| NCT02587689 | MUC1 | I/II | 20 | Company | Unknown |  |
| NCT03013712 | EpCAM | I/II | 60 | Academic | Recruiting |  |

1Location of study is United States, all other trials are conducted in China. HAI: Hepatic artery infusion; IT: Intratumoral injection; IV: Intravenous injection; TACE: Transarterial chemoembolization.

**Table 4 Available techniques for induction of hepatocellular carcinoma in relation to temporal and technical aspects as well as major advantages and disadvantages (summarized from[92])**

|  |  |  |  |
| --- | --- | --- | --- |
| **Method and Specification** | **Time to HCC**  **short (+) to long (+++)** | **Technical efforts**  **low (+) to high (+++)** | **Major “Pros” (+) *vs* “Contras” (-)** |
| Chemotoxic agents linked models |
| Diethylnitrosamine | ++ | + | (+) good combination options with other methods |
| 9,10-dimethyl-1,2-benzanthracene | (-) time to HCC not easily predictable |
| Direct implantation of tumor cells or tissue |
| Heterotopic/Orthotopic | + | +/++ | (+) heterotopic xenografts are often and easily done (+) syngeneic orthotopic models better reflect the natural liver microenvironment |
| Syngeneic/Xenografts | (-) xenografts need immunocompromised mice (-) orthotopic tumor implants need surgical and imaging experience |
| Genetically engineered mouse models |
| Mouse embryo manipulation | ++/+++ | +++ | (+) hepatocarcinogenesis can be analyzed stepwise  (-) effects of manipulated gene(s) could have heterogeneous latency and genetic penetrance |
| Cre-Lox recombination |
| Hydrodynamic injection |
| CRISPR-Cas9 |
| Humanized mouse models |
| Immunologically humanized mice | +++ | +++ | (+) immunotherapeutical issues can be studied based on human cell lines in mice  (-) establishment difficult due to engraftment failure and development of stable stem cell-derived hepatocytes |
| Genetically humanized mice |

HCC: Hepatocellular carcinoma.